DEVELOPMENT, CHARACTERIZATION AND EVALUATION OF MESALAMINE LOADED PROBIOTIC BASED MICROCARRIERS FOR THE MANAGEMENT OF ULCERATIVE COLITIS

A

RESEARCH PROPOSAL

SUBMITTED

IN PARTIAL FULFILLMENT TO THE REQUIREMENT

FOR

DOCTOR OF PHILOSOPHY

IN

FACULTY OF PHARMACY

DISCIPLINE: PHARMACEUTICS

By

Mr. Amandeep Singh
Enrollment No: 17201MPE02

MAHARAJA RANJIT SINGH PUNJAB TECHNICAL UNIVERSITY

BATHINDA (INDIA)

(October 2020)

CONTENTS

- TITLE
- INTRODUCTION
- LITERATURE REVIEW
- RESEARCH ISSUES
- PROBLEM FORMULATION
- RESEARCH OBJECTIVES
- NEED OF PROPOSED WORK
- METHODOLOGY
- BIBLIOGRAPHY
- PLACE OF WORK
- RESOURCES REQUIRED
- EXTENT OF EXTERNAL ASSISTANCE

1. PROPOSED TITLE: DEVELOPMENT, CHARACTERIZATION AND EVALUATION OF MESALAMINE LOADED PROBIOTIC BASED CARRIERS FOR THE MANAGEMENT OF ULCERATIVE COLITIS

2. INTRODUCTION: ULCERATIVE COLITIS

Inflammatory bowel diseases (IBD) is chronic idiopathic relapsing inflammatory disease of the gastrointestinal tract (GIT) tract, Generally IBD is divided into two categories, Ulcerative colitis (UC) and crohn's disease (1). In case of crohn's disease inflammation can occur in any part of digestive system (Mouth to anus), but in case of UC inflammation occurs only in ascending or descending parts of colon. UC is chronic and relapsing disease of the colon, characterized by inflammation with ulcer formation in the lining of colon. A basic symptom of ulcerative colitis includes abdominal pain, blood in stool, weight loss, fatigue and severe pain in the rectum (2). Severe form of ulcerative colitis leads to colon cancer. Chronic inflammation generated by the production of reactive oxygen species leads to generate dysplasia, which further turns into colitis associated colorectal cancer (3). Therefore, there is the high risk of colon cancer in patients with ulcerative colitis (4).

2.1 Morbidity and mortality of ulcerative colitis

The incidence of UC from Punjab was investigated by cluster sampling method. A population of 51,910 people was screen, among which two thirds lived in rural parts of Punjab and the rest in urban parts. Overall, 23 patients were diagnosed with UC leading to a prevalence rate of 44.3/100,000. These data again indicate that UC is not rare in India (5). However, the crude overall incidence of IBD, UC, and CD from Asia was 1.37, 0.76, and 0.54 per 100,000, respectively, whereas in Australia it was 23.67, 7.33, and 14.00, respectively. Among the Asian countries, it was highest in mainland China (Guangzhou: 3.4/ 100,000) followed by Hong Kong (3.06/100,000), and Macau (2.2/100,000). Data from Western Asia including studies from Kuwait, Turkey and Israel. Revealed an incidence rate of 2.8/100,000 and 5.04/100,000 in Kuwait and Israel, respectively, and a prevalence of 4.9/100,000 and 167/100,000 in Turkey and Israel, respectively (6, 7). All around the globe, UC is turning out to be a global disease, because of increasing incidences and prevalence’s of it with respect to time.

2.2 Conventional treatment strategies for ulcerative colitis

The treatment of Ulcerative Colitis depends upon the severity of disease. The most commonly used drugs for its treatment are anti-inflammatory agents which mainly include 5-amino salicylates like mesalazine ,olsalazine and balsalazide, which are used for the cure of mild to moderate attacks and to maintain remission in UC. Some immunosuppressive agents such as azathioprine, 6-mercaptopurine, methothexate, cyclosporine, and tacrolimus are routinely prescribed by doctors. Other classes of drugs which can also be used are corticosteroids, like prednisolone and anti-TNF-α-antibodies for moderate to severe conditions of Ulcerative Colitis (8).

2.3 Selection of drug: Mesalamine is first line choice of drug for maintaining remission of mildly to moderately active ulcerative colitis. Mesalmine is frequently prescribed by the physician. In ulcerative colitis reactive oxygen species and inflammatory mediator played an important, mesalmine act by decreasing the ROS formation and decreasing the concentration of inflammation mediators. Sulfasalazine (SFZ) is also used in the treatment of UC for decades; intolerance to the sulfapyridine moiety of sulfasalazine is quite common. A large number of patients intolerant to sulfasalazine are able to tolerate mesalamine; Glucocorticoids like Beclomethasone are also used for the treatment of UC but cause weight gain and also effecting the immunity system.The previous data suggested that mesalmine is good choice of drug for the management of ulcerative colitis(9).

2.4 Mesalamine: In conventional treatment of ulcerative colitis mesalamine is first line choice of drug available in tablet (Mesacol), capsule (Pentasa), rectal enemas (Perrigo). It is rapidly absorbed from the small intestine but fails to reach into the colon site. Furthermore, long term medication produces lots of side-effects, which negatively affect the quality of life of ulcerative colitis suffered patients. Additionally, poor targeting of orally administered drug and low adherence to desired site represent frequent therapeutic challenges. In case of enemas, patient may not feel comfort as with the rectal route, chances of cloth staining is more. Mesalmine is belongs to a class of drugs known as aminosalicylate having Anti-inflammatory and Anti-oxidant property (9, 10). It works by decreasing swelling in the colon and reduces the IL-2

cytokines production in peripheral mononuclear cells and thereby inhibiting T-cell proliferation, altering cell adhesion expression pattern, inhibiting antibody production and mast cell release, and interfering with macrophage and neutrophil, 5-ASA may also decrease IL-1 and TNF-α, induce apoptosis of lymphocytes and regulate NF-kB, mesalazine decrease inflammation by blocking cyclooxygenase and inhibiting prostaglandin production in the colonic region (11).

3. Probiotics role in ulcerative colitis.

Probiotics are non digestible live microorganisms, when given an indefinite quantity, provides health welfare to the host. The colon contains over 400 distinct species of bacteria having a population of 10^{11}–10^{12} CFU (Colony Forming Unit) ml^{-1} with Bacteoides, Bifidobacterium, Eubacterium and Lactobacillus greatly out numbering other species. UC is a mucosal disease; probiotic therapy which works on mucosal level might be beneficial. Probiotics acts by several mechanisms, Firstly probiotics act a safe barrier, and they cover the intestinal tract near to the brush border and through competitive inhibition, and do not allow the luminal bacteria from reaching the lamina propria and stimulating the mucosal immune system. Secondly, probiotoic enhances the production of mucous and probiotics can modify the consistency of the mucus, thus changing bacterial adherence patterns and act as protective against bacteria thirdly, probiotics cause the mucosal immune system to secrete the protective immunoglobulin's (Ig) such as secretory IgA and a host of protective bacteriocins and defensins into the lumen. Ultimately, probiotics change the function of the mucosal immune system to make it more anti-inflammatory. This mechanism appears to be particularly important in UC. With these all mechanism probiotics can diminish the effect of luminal bacteria which are responsible for inflammation. Administration of probiotics may recover the commensal microflora and normalise the host-microbial interaction (12). Certain types of bacteria have probiotic properties. They essentially function to alter the type and amount of bacteria in the gastrointestinal tract and therefore, positively manipulate the enteric flora. They have both local and systemic effects, such as to regulate the host immune response and improve the epithelial barrier function by decreasing intestinal permeability and enhancing tight junctions. Probiotics work for a healthier gut, act by indirectly enhancing the number of fatty acids with short

chains, which lower colonic pH and thus help to stop the development of pathogenic bacteria. The short chain fatty acids butyrate provides nutrition to the intestinal epithelium. Co-administration of probiotics along with the drug delivery system refurbishes the colonic micro-flora which had been damaged or lost due to effect of the drug as well as pathophysiological conditions in UC (13).

3.1 Selected probiotic strain:-Saccharomyces boulardii: It is a nonpathogenic strain of Yeast, Saccharomyces boulardii has probiotic properties and it is also the only yeast approved as a human probiotic. There are several controlled studies showing that S. boulardii has a positive effect in diseases like ulcerative colitis, antibiotic- associated diarrhea, traveler's diarrhea, acute gastroenteritis (13).

3.2 Reason for combination of mesalamine with probiotic:

The oral administration of probiotic results in a large loss of viability associated with passage through the stomach, this leads to loss of viability and efficacy. By encapsulating the probiotic with carriers (Microparticle and pellets), will able to protect the probiotic from harsh acidic medium and provides maximum beneficial effect in diseased colon site.

4 Polymer selections:

Proposed polymers:

4.1 Coated polymer:

4.1.1 -Cellulose acetate phthalate (CAP): CAP is a commonly used polymer phthalate in the formulation of pharmaceuticals, such as coating of tablets or capsules and for controlled release formulations and a enteric coated polymer . It prevents the release of drug from acidic environment (14).

4.2 Enzyme based polymer:

4.2.1 -Pectin:

Pectin, a natural heteropolysaccharide, is characterized with 1,4 linked a-D-galactosyluronic acid residues and neutral sugars such as rhamnose, galactose and arabinose. It is popularly used as colon- specific drug delivery vehicle for colon disease. It has positive effect on ulcerative colitis disease state. Pectin can be selectively digested by colonic microflora to induce site-specific drug release, and has a strong tendency to interrupt the colon tumor growth biochemically. The pectin is an

aqueous soluble polymer and it undergoes partial degradation at gastric pH 2–4 via side chain hydrolysis and at small intestinal pH 5–6 via b-elimination of main chain or de-esterification (15), (16). The matrix of pectin tends to swell, erode and has premature drug release at upper gastrointestinal tract. The colon- specific oral dosage forms are designed chiefly through coating of drug matrix with cellulose acetate phthalate.

4.3 Proposed strategies:

4.3.1Enteric Coated and enzyme based strategies

We will prepare pectin based formulation (micro particles and pellets) followed by coating with CAP. The Coated CAP will prevent the drug release in stomach. Furtherly, at pH in the range of 6.0 to 7.4 CAP layer will dissolve and come in contact with colon's specific enzymes (Bacteroids Bifidobacterium, Eubacterium) and start releasing the drug (Mesalamine) and probiotic (*Saccharomyces boulardii*) at inflammed colon site. The above said strategy has chosen to avoid burst drug and probiotic release in the stomach.

4.4 Proposed carriers for colon drug delivery:

4.4.1. Microparticle as carrier:

The microparticle is polymer matrix with an active moiety dispersed inside free flowing spherical particles. The active ingredients in the matrix is usually dissolved or melted in polymer before processing. It provides constant and prolonged therapeutic effect. Microparticle carrier system has the ability to provide specific uptake in certain areas, such as absorption in the colon, in order to target inflammation sites in UC. The particle size of microparticle is ranging from 1-1000 μm. In conventional dosage form the major drawback of mesalamine is rapidly absorbed from the small intestine and showed systemic bioavailability which further shows unwanted effect of drug. Therefore it is necessary to develop a colon-specific delivery system for mesalamine so that drug can be targeted to inflammation site of colon. Microparticles could be best approach for colon targeting. The particle size 5–15 μm of enzyme based microparticle provides localization of drug, ultimately increases the residence time of drug in the colonic region. Particle size of this range is able to adhere at the site of inflammation, finally provide maximum release of drug at colonic pH with maximum pharmacological action (17).

4.4.2 Pellets as carrier :

Pellets are solid, small free flowing spherical units, prepared by agglomeration of fine powder called pellets. Pellets range in size typically, between 0.5 – 1.5 mm, though other sizes could be prepared. Bulking agents and binders are required for preparation of pellets by extrusion and spheronization method. We are using pectin as a core material binder agent , and MCC is used as bulking agent. CAP as coating polymer for colon targeted drug delivery. CAP will prevent the drug release from gastric acidic medium. Polysaccharides polymers such as guar-gum, pectin are used in colon targeted drug delivery systems due to its drug release retarding property and susceptibility to microbial degradation in large intestine. The mixture of probiotic with mesalamine and polysaccharide polymer with necessary diluent will be allowed to form pellets by extrusion and spheronization method (18).

4.4.3 Reason for preparing both microparticle and pellets as carrier: The composition of microparticle and pellets are different. Their *in-vivo* effect with respect influence of drug release and *in-vivo* drug targeting might be different which may further influence therapeutic efficacy.

5. Literature review:

- Elizaberth Managalia *et al*. (19) Suggest that Mesalamine, 5-aminosalicylic acid (5-ASA), is a potent antioxidant and is known to enhance peroxisome proliferator–activated receptor activity in the intestine. Reactive oxygen species in colonic progenitor cells and enhances PTEN (Phosphatase and tensin homolog) activity, thus attenuating PI3K/Akt signaling. These data suggest that the antioxidant properties of 5-ASA may be the predominant mechanism for 5-ASA chemoprevention.
- Nidhi *et al.*(20) has developed embelin loaded guar gum microparticles through emulsification technique. The in vitro release of the optimized formulation was found to be 88.5±3.8% in 24 hr, have exhibit remarkably sustain and delayed the release of embelin at a specific site. Embelin loaded microparticles showed average particle size of 12.9± 0.75 μm. This approach produced comparatively less side-effect to another conventional dosage form. The drug release was site-specific and sustained. This approach produced less side-effect as compare to another conventional dosage form.

The study examined the application of the microparticulate system in enhancing stability and controlled release.

- Seema Badhana *et al*.,(21) prepared microspheres by the ionic-gelation emulsification method using tripolyphosphate (TPP) as cross linking agent. The microspheres were coated with Eudragit S-100 by the solvent evaporation technique to prevent drug release in the stomach. It was observed that Eudragit S-100 coated chitosan microspheres gave maximum release of drug in the colonic environment. Eudragit-coated chitosan microspheres were promising carriers for colon-targeted delivery of Mesalamine.
- Ankita Sharma *et al.*(22) has developed embelin loaded guar gum microparticles through emulsification technique. The in vitro release of the optimized formulation was found to be 88.5±3.8% in 24 hr, have exhibit remarkably sustain and delayed the release of embelin at a specific site. Embelin loaded microparticles showed average particle size of 12.9± 0.75 μm.This approach produces comparatively less side-effect to another conventional dosage form. The drug release is site-specific and sustained. This approach produces less side-effect as compare to another conventional dosage form. The study examined the application of the microparticulate system in enhancing stability and controlled release.
- Varshosaz J *et al.*(23) has prepared colon-specific pellets of budesonide, using pectin as film coating. Pellet cores of budesonide were prepared by extrusion / spheronization technique. Pectin, in different ratios was combined with Eudragit RS30D, Eudragit NE30D or Surelease to produce film coating. The dissolution profiles of pectin coated pellets were investigated in pH of 1.2 (2 h), pH of 7.4. (4 h) and pH of 6.8 in the absence as well as presence of rat cecal contents (18 h). This confirmed the ability of the optimized formulation for targeted drug delivery of budesonide to colon.
- Sureshkumar R *et al.* (24) High molecular weight hydroxypropyl methylcellulose (HPMC) and biodegradable pectin were used for coating the pellets containing curcumin, to be released in the colon. The prepared pellets were free flowing. *In-vitro* release of curcumin remained intact up to pH 3.0, disintegrated at pH 7.2, and released up to 12 hours. The ideal batch (1:3) showed minimum release at pH 1.2 and maximum release at pH 6.8, and an increased amount of curcumin in the blood stream(1.287 μg/ml) was achieved when compared with pure curcumin (0.5 μg/ml)

- Fan L *et al.*(25) investigated the physiochemical characteristics and preventive effects of purified pectin (H121, L13 and L102) with different esterification degrees on dextran sulfate sodium (DSS)-induced colitis in mice. Three doses of each type of pectin were administered to C57BL/6J mice for 7 days before the DSS treatment, with dextran and Mesalazine as positive controls. The results suggested that pectin could attenuate DSS-induced intestinal epithelial injury, inflammation and oxidative stress.
- Avachat AM *et al.*(26) **Viability count and release of saccharomyces boulardii in the formulations during dissolution**- The viability of S.b. was checked along with its release in the co-formulated Vancomycin HCl and S.b. pellets for 12 h. Ten milliliters of aliquots were withdrawn from the dissolution apparatus. One milliliter of sample was taken from withdrawn aliquots. The resulting solution was considered as a stock solution (10^{-1}). From this solution, 1ml was diluted with sterile 0.1% peptone+0.05% Tween 80 solution up to 1ml to obtain 10^{-3} dilution. These serial dilutions of these aliquots were made until a suitable colony count, i.e. 10^{-7} was obtained. The cell suspension was then transferred into the petri plates and then 15–20 ml soyabean casein digest agar media was poured in to each plate. The plates were further incubated at 29±1°C under aerobic conditions for 96–120 h. This plating procedure was carried out in triplicate. Colonies of S.b. were counted and results were expressed as log CFU/g (colony forming units per gram) of yeast. All the viability and probiotic release studies were carried out on six capsules (filled with pellets) for each batch.
- **Loading dose of probiotic and ways to load probiotic in the formulation need to be properly addressed.**
  The dose of lyophilised Saccharomyces *boulardii* is (10^{9}) cfu/ml. We will also consider this dose for our research work. Complete process has been described in figure-1.

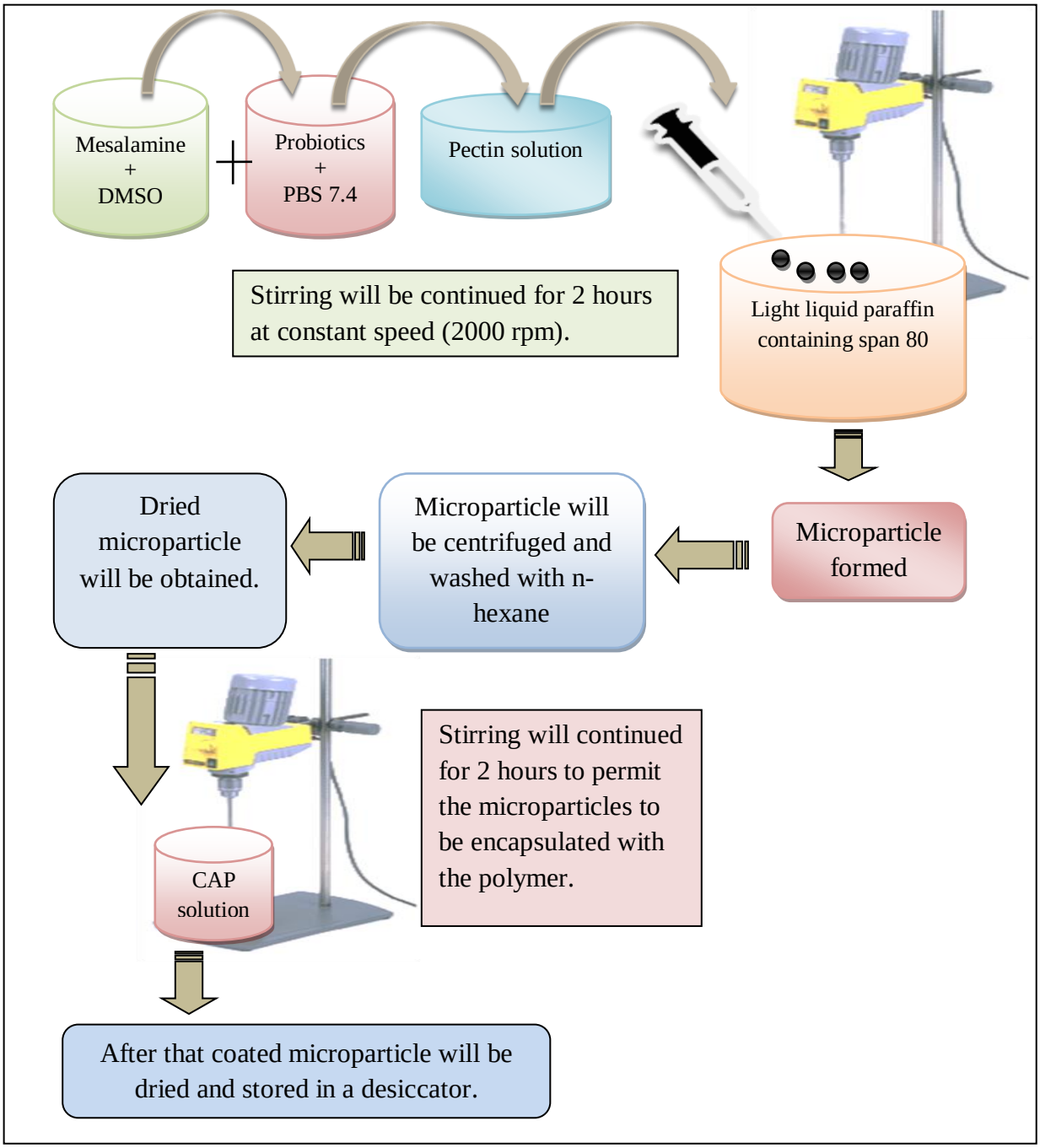
Mesalamine
+
DMSO
Probiotics
+
PBS 7.4
Pectin solution
Stirring will be continued for 2 hours at constant speed (2000 rpm).
Light liquid paraffin containing span 80
Microparticle formed
Microparticle will be centrifuged and washed with n-hexane
Dried microparticle will be obtained.
CAP solution
Stirring will continued for 2 hours to permit the microparticles to be encapsulated with the polymer.
After that coated microparticle will be dried and stored in a desiccator.

Figure-1:- Way to load probiotic in the formulation:- Mesalamine and probiotic loaded microparticles will be prepared by emulsification technique.

10^9 cfu/ml suspension of lyophilised probiotics will be prepared in PBS 7.4

Extrusion– spheronization instrument

Pellets will be produced by powder blend containing mesalamine, pectin, HPMC an MCC in a mortar by mixing it properly.

Then , q.s water will be added to above mixture to moist the powder to form proper extrude.

Then it will mix well all the ingredients with water in mortar.

Add above mixture into the extrude inlet slowly.

Extrusion

Then the extrude will be spheronised in spheronizer at different speeds and time intervals

Pellets formed

Figure-2 Way to load probiotic in the formulation:- Mesalamine and probiotic loaded pellets will be prepared by extrusion– spheronization method.

6. Research issue: Ulcerative colitis is an autoimmune**,** chronic idiopathic, inflammatory relapsing disease of colon; So long term management strategy is required. In case of conventional treatment strategy drugs including mesalamine, the first line treatment choice of drug is rapidly absorbed from the small intestine with 20-30 % oral bioavailability and drug is not reached to colonic site.

7. Problem formulation

Due to rapid absorption of mesalamine from the small intestine, the systemic bioavailability of mesalmine shows many side effects like hepatotoxicity cramping, headache, nausea, aching and vomiting this is happen due to the non specific targeted delivery of mesalamine to the colonic region. So we need to design a carrier which will be able to release the drug in controlled manner particularly in colon site with maximum bioavailability. So that will helps in appropriate local targeting and decreases drug related side-effects.

8. Research Aim: The main aim of the current study is to design, develop and evaluate novel drug delivery systems (microspheres and microcapsules) containing mesalamine and probiotic strain at colon site to ensure optimum anti-inflammatory and achieve increased therapeutic effectiveness and least extent of side effects.

9. Research objective: In order to achieve the aim of this research, the following objectives are proposed:

1. To develop microparticulate formulations of mesalamine with probiotics.
2. .To optimizes critical variables (materials) for development of microparticulate formulations.
3. To screen and select best strain of probiotics based on anti-oxidant and anti-inflammatory properties.
4. To carry out accelerated studies of optimized formulations.
5. To evaluate *in-vivo* efficacy and pharmacokinetics of developed formulations.

10. Need of proposed work

To overcome the drawbacks of conventional dosage forms, formulation development is aimed to design for targeted delivery of drug and probiotic to inflammation site with enhanced activity, reduced dosing frequency for improved therapeutic benefit to ulcerative colitis patients.

10.1 Novelty: Novelty of the strategy lies in two concepts for effective treatment strategy of UC:

1. Appropriate targeting of drug and probiotic therapy to the affected colonic area and selection of most effective drug augmented with addition of probiotic to get synergistic action of anti-inflammatory and antioxidant

2. The same has not been reported in the literature so far. Concept of targeting is also unique by coating with core of CAP and followed by enzyme based approach. Till now no *in-vivo* study is reported by using these probiotic strain combination with probiotic. No commercial product with mesalamine and probiotics are available in market.

11. Methodology:

11.1 Proposed Method used for preparation of microparticle:

(a) Emulsification method
(b) Solvent evaporation method

11.2 Proposed Method used for preparation of pellet.

(a) Extrusion and spheronization method
(b) Hot melt Extrusion.

12. Plan of work

12.1 Preformulation studies

a) Drug Morphology (Odour,Apperance)
b) Physical state (DSC)
c) Melting point
d) Partition coefficient
e) Solubility study
f) Compatibility Study (FTIR & DSC)

12.2 Analytical method development and validation

a) UV spectrophotometric method for *in-vitro*
b) HPLC Method for drug stability assay

13. Selection of probiotic strain on the basis of antioxidant property

14 Preparation and optimization by DOE & RSM

14.1 Microparticle formulation

a) **Independent variables:** Drug: polymer, Surfactant concentration, Stirring speed, Concentration of cross linker. We will initially test these independent variables; out of these we will select two to three variables whose effects are predominant

b) Dependent (Response variables): Particle size, Entrapment efficiency, in-*vitro* drug release

14.2 Pellets formulation

a) **Dependent variables:** Particle size, Entrapment efficiency, *in-vitro* drug release

b) **Independent variables:** Drug: polymer, Binder, Bulking agent, Rotation speed of spheronizer and extruder (RPM), Rotation time. We will initially test these independent variables; out of these we will select two to three variables whose effects are predominant

15. *In-vitro* characterization of optimized formulation of microparticle.

a) Loading efficiency

b) Drug entrapment efficiency

c) Particle morphology (SEM)

d) Particle size analysis , polydispersity index, Particle charge

e) Zeta potential

f) *In-vitro* drug and probiotic release

g) FT-IR spectroscopy

h) XRD

i) DSC

j) Cell line study (Caco-2 cell line)

k) Storage stability studies

16. *In-vitro* characterization of optimized formulation of pellet.

a) Loading efficiency

b) Drug entrapment efficiency

c) Particle morphology (SEM)

d) Sphericity studies(optical microscopic)

e) Micrometric properties (Angle of repose, Bulk density, Tapped density, Hausner's ratio, Carr’s index and friability.

f) *In-vitro* drug and probiotic release

g) FT-IR spectroscopy

h) XRD

i) DSC

j) Cell line study (Caco-2 cell line)

k) Storage stability studies

18. Induction of ulcerative colitis in Wistar rat

By TNBS model: TNBS is 2, 4, 6-trinitrobenzene sulfonic acid, basically it is the model for induction of ulcerative colitis in animal (Wistar rat).

Group distribution for Comparison of activity:

1st Group - Normal control (No disease)

2nd Group -TNBS control (Disease)

3rd Group - Placebo group (Inert material formulation like HPMC)

4th Group- Pectin plain formulation

5th Group- Mesalamine formulation

6th Group- Probiotic formulation

7th Group- Mesalamine+ probiotic formulation

19. *In-vivo* studies

a) Morphological analysis

b) Histopathological studies

c) Oxidative stress studies

   a) Determination of Myeloperoxidase (MPO) colonic activity
   b) Determination of Lipid peroxidation(LPO) colonic activity
   c) Determination of Glutathione(GSH) colonic activity

d) Identification of Serum-Markers

   a) C-reactive protein
   b) Erythrocyte sedimentation rate (ESR)
   c) White blood cell count

e) Pharmacokinetic studies for both formulation (Microparticle and pellet)

20. Statistical analysis: ANOVA-Analysis of variance followed by post hoc tukey test, if required.

21. Bibliography:

1. Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. The Journal of clinical investigation. 2007 Mar 1;117(3):514-21.

2. Rashid M, Kaur V, Hallan SS, Sharma S, Mishra N. Microparticles as controlled drug delivery carrier for the treatment of ulcerative colitis: A brief review. Saudi Pharmaceutical Journal. 2016 Jul 1;24(4):458-72
3. Rogler G. Chronic ulcerative colitis and colorectal cancer. Cancer letters. 2014 Apr 10;345(2):235-41.
4. Navaneethan U, Jegadeesan R, Gutierrez NG, Venkatesh PG, Hammel JP, Shen B, Kiran RP. Progression of low-grade dysplasia to advanced neoplasia based on the location and morphology of dysplasia in ulcerative colitis patients with extensive colitis under colonoscopic surveillance. Journal of Crohn's and Colitis. 2013 Dec 15;7(12):e684-91.
5. Kedia S, Ahuja V. Epidemiology of inflammatory bowel disease in India: the Great Shift East. Inflammatory intestinal diseases. 2017;2(2):102-15.
6. Ray G. Long-term maintenance treatment of ulcerative colitis in eastern India: a 15-year follow-up. Tropical doctor. 2018 Jul;48(3):207-13.
7. Ng SC, Tang W, Ching JY, Wong M, Chow CM, Hui AJ, Wong TC, Leung VK, Tsang SW, Yu HH, Li MF. Incidence and phenotype of inflammatory bowel disease based on results from the Asia-pacific Crohn's and colitis epidemiology study. Gastroenterology. 2013 Jul 1;145(1):158-65.
8. Kucharzik T, Asthana AK. Medical Management 2: Conventional. InInflammatory Bowel Disease Nursing Manual 2019 (pp. 81-90). Springer, Cham.
9. Rizzello F, Salice M, Calabrese C, Mazza M, Calafiore A, Calandrini L, Hrustemovic HP, Campieri M, Gionchetti P. Medical Treatment of Ulcerative Colitis: Does Traditional Therapy Still Have a Role?. InUlcerative Colitis 2019 (pp. 93-104). Springer, Milano.
10. Managlia E, Katzman RB, Brown JB, Barrett TA. Antioxidant properties of mesalamine in colitis inhibit phosphoinositide 3-kinase signaling in progenitor cells. Inflammatory bowel diseases. 2013 Jul 17;19(10):2051-60
11. Tripathi K, Feuerstein JD. New developments in ulcerative colitis: latest evidence on management, treatment, and maintenance. Drugs in context. 2019;8.
12. Zipporah IE, Lakhbir K, Morris G, Patricia B, Anthony A. P002 The use of probiotics in the maintenance of remission in ulcerative colitis: A systematic review. American Journal of Gastroenterology. 2019 Jul 1;114:S1.

13. Floch MH. The role of prebiotics and probiotics in gastrointestinal disease. Gastroenterology Clinics. 2018 Mar 1;47(1):179-91.
14. Kapoor D, Maheshwari R, Verma K, Sharma S, Ghode P, Tekade RK. Coating technologies in pharmaceutical product development. In Drug Delivery Systems 2020 Jan 1 (pp. 665-719). Academic Press.
15. Noreen A, Akram J, Rasul I, Mansha A, Yaqoob N, Iqbal R, Tabasum S, Zuber M, Zia KM. Pectins functionalized biomaterials; a new viable approach for biomedical applications: A review. International journal of biological macromolecules. 2017 Aug 1;101:254-72.
16. Kang RK, Mishr N, Rai VK. Guar Gum Micro-particles for Targeted Co-delivery of Doxorubicin and Metformin HCL for Improved Specificity and Efficacy Against Colon Cancer: In Vitro and In Vivo Studies. AAPS PharmSciTech. 2020 Feb;21(2):1-1.
17. Rashid M, Kaur V, Hallan SS, Sharma S, Mishra N. Microparticles as controlled drug delivery carrier for the treatment of ulcerative colitis: A brief review. Saudi Pharmaceutical Journal. 2016 Jul 1;24(4):458-72.
18. Singh MN, Hemant KS, Ram M, Shivakumar HG. Microencapsulation: A promising technique for controlled drug delivery. Research in pharmaceutical sciences. 2010 Jul;5(2):65.
19. Managlia E, Katzman RB, Brown JB, Barrett TA. Antioxidant properties of mesalamine in colitis inhibit phosphoinositide 3-kinase signaling in progenitor cells. Inflammatory bowel diseases. 2013 Jul 17;19(10):2051-60.
20. Nidhi, Dadwal A, Hallan SS, Sharma S, Mishra N. Development of enteric-coated microspheres of embelin for their beneficial pharmacological potential in ulcerative colitis. Artificial cells, nanomedicine, and biotechnology. 2017 Aug 18;45(6):1092-100
21. Badhana S, Garud N, Garud A. Colon specific drug delivery of mesalamine using eudragit S100-coated chitosan microspheres for the treatment of ulcerative colitis. International Current Pharmaceutical Journal. 2013 Feb 1;2(3):42-8.
22. Sharma A, Kaur N, Sharma S, Sharma A, Rathore MS, Ajay K, Mishra N. Embelin-loaded guar gum microparticles for the management of ulcerative colitis. Journal of microencapsulation. 2018 Feb 17;35(2):181-91.

23. Varshosaz J, Emami J, Tavakoli N, Minaiyan M, Rahmani N, Dorkoosh F, Mahzouni P. Pectin film coated pellets for colon-targeted delivery of budesonide: In-vitro/in-vivo evaluation in induced ulcerative colitis in rat. Iranian journal of pharmaceutical research: IJPR. 2012;11(3):733.
24. Sureshkumar R, Munikumar M, Ganesh GN, Jawahar N, Nagasamyvenkatesh D, Senthil V, Raju L, Samantha MK. Formulation and evaluation of pectin-hydroxypropyl methylcellulose coated curcumin pellets for colon delivery. Asian Journal of Pharmaceutics (AJP): Free full text articles from Asian J Pharm. 2014 Sep 24;3(2).
25. Fan L, Zuo S, Tan H, Hu J, Cheng J, Wu Q, Nie SP. Preventive effects of pectin with various degrees of esterification on ulcerative colitis in mice. Food & Function. 2020.
26. Avachat AM, Shinde AS. Feasibility studies of concomitant administration of optimized formulation of probiotic-loaded Vancomycin hydrochloride pellets for colon delivery. Drug development and industrial pharmacy. 2016 Jan 2;42(1):80-90.

22 Place of Work: Department of pharmaceutics I.S.F. College of Pharmacy, Moga (Punjab.)

23 Resources (Hardware/Software/) required & availability status: All required glassware's, chemicals and equipment are available at the place of work.

24 Extent of External Assistance Required (if any): for TEM, SEM and DSC

Submitted by:

Mr. AMANDEEP SINGH
Research scholar

Supervisor:

DR. R K NARANG
Professor in department of Pharmaceutics
I S F College of Pharmacy,
Moga (Punjab)

Co-Supervisor:

DR. UTTAM KUMAR MANDAL

Associate Professor in department of Pharmaceutics

MRSPTU, Bathinda (Punjab)